REMARKS

I. STATUS OF THE CLAIMS

Upon entry of this amendment, claims 1-66 are pending in this application and are presented for examination. Claim 64 has been amended to correct a typographical error. As such, no new matter has been introduced with the foregoing amendment. Reconsideration is respectfully requested.

II. REJECTION UNDER 35 U.S.C. § 102(a)

The claims have been rejected in various combinations under 35 U.S.C. § 102(a) over a number of different references. Each of these rejections is traversed in detail below.

For a rejection of claims under § 102 to be properly founded, the Examiner must establish that a single prior art reference either expressly or inherently discloses each and every element of the claimed invention. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Verdegaal Bros. V. Union Oil Co. Of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

In *Scripps Clinic & Research Found. v. Genentech, Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991), the Federal Circuit held that:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found with a single prior art reference. . . . There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Id.* at 1010.

Anticipation can be found, therefore, only when a cited reference discloses all of the elements, features, or limitations of the presently claimed invention.

A. Lee *et al.* I

Claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Lee *et al.* (U.S. Patent No. 5,908,777) ("Lee *et al.* I"). Applicants respectfully traverse.

The Examiner alleges that Lee *et al.* I discloses compositions containing a condensed nucleic acid encapsulated within a liposome (*see,* Office Action at page 2). In response, Applicants assert that Lee *et al.* I fails to teach all of the elements of the claimed invention.

As previously explained by Dr. Ian MacLachlan in his Declaration under 37 C.F.R. § 1.132 submitted on September 30, 2005, Lee *et al.* I discloses nucleic acid-lipid ***complexes*** comprising anionic liposomes and nucleic acid-polylysine complexes formed by mixing preformed liposomes with nucleic acid-polylysine complexes in deionized water (*see,* Declaration at ¶ 9). As Dr. MacLachlan clarifies, given that DNA does not readily cross lipid membranes, one of skill in the art would appreciate that mixing a nucleic acid-polylysine complex with preformed liposomes in an aqueous solution does not result in entrapment of DNA within the internal space of the liposomes, but would, instead, result in the formation of nucleic acid-lipid ***complexes*** (*see,* Declaration at ¶ 9). Without a step that destabilizes the liposome membrane, the nucleic acid would not be able to enter the liposome and be encapsulated (*see,* Declaration at ¶ 9). Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid ***complexes*** of Lee *et al.* I do ***not*** comprise a nucleic acid fully encapsulated in a liposome (*see,* Declaration at ¶¶ 9 and 18). Accordingly, Lee *et al.* I does not anticipate the presently claimed invention.

In view of the foregoing remarks, Applicants urge the Examiner to withdraw this aspect of the rejection under 35 U.S.C. § 102(a).

B. Martin *et al.*

Claims 1-6, 8, 12-13, 15-17, 21-22, 28, 32-37, 39, 43-45, 49, 55, 57, and 59 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Martin *et al.* (U.S. Patent No. 5,891,468). Applicants respectfully traverse.

The Examiner alleges that Martin *et al.* discloses compositions containing a condensed nucleic acid encapsulated within a liposome (*see,* Office Action at page 4). In response, Applicants assert that Martin *et al.* fails to teach all of the elements of the claimed invention.

As previously explained by Dr. MacLachlan, Martin *et al.* discloses ***complexes*** formed by mixing preformed liposomes with plasmid-histone complexes (*see,* Declaration at ¶ 10). Thus, as Dr. MacLachlan has clarified, Martin *et al.* does not describe nucleic acid-histone complexes fully encapsulated in a liposome (*see,* Declaration at ¶ 10).

Dr. MacLachlan further clarifies that the dehydration-rehydration-extrusion methods described in Martin *et al.* cannot be used to encapsulate nucleic acids (*see,* Declaration at ¶¶ 10 and 16). Specifically, Dr. MacLachlan describes experiments conducted under his supervision that use dehydration-rehydration-extrusion methods to attempt to encapsulate nucleic acids in liposomes (*see,* Declaration at ¶ 16). The experiments described by Dr. MacLachlan were conducted as follows. First, a lipid solution containing a total of 2.22 μmoles lipid and comprising DOPE:DODAC:PEG-ceramide C14 (82.5:7.5:10 molar percent) was prepared by dissolving the lipids in chloroform and using nitrogen gas to drive off chloroform to form a lipid film. The lipid film was then hydrated with 2 ml phosphate buffered saline (pH 7.4) containing 50 or 100 μg of nucleic acid (*i.e.*, plasmid DNA) to generate liposomal samples with drug (*i.e.*, nucleic acid):lipid ratios of 22.5 and 45 μg input DNA/μmol lipid. The resulting suspension was subjected to 5 rounds of freezing in liquid nitrogen and thawing in a 37°C water bath to increase the homogeneity of the resulting multilamellar vesicles, which were all greater than 10,000 nm in diameter. To produce liposomes of appropriate size, the samples were then extruded 10 times through 2 stacked 100 nm polycarbonate filters using a 10-mL Extruder (Northern Lipids Inc.) and nitrogen gas at 400-600 psi. Nucleic acid encapsulation was determined using membrane-impermeable Picogreen, which fluoresces in the presence of plasmid DNA. The proportion of nucleic acid encapsulated in the liposomes was determined by measuring the fluorescence intensity of the Picogreen before and after the addition of the detergent Triton X-100 (*see,* Declaration at ¶ 16).

The results from the experiments are set forth in Exhibit B accompanying the Declaration and demonstrate that plasmid encapsulation and recovery were both extremely inefficient at both of the input nucleic acid amounts examined. Specifically, as Dr. MacLachlan explains, prior to extrusion, only 12% or 15% of the input nucleic acid was inaccessible to

Picogreen due to its association with or incorporation into >10,000 nm multilamellar vesicles (*see*, Declaration at ¶ 16 and Exhibit B). In addition, only 1.4% or 2% of the input nucleic acid was actually recovered after the extrusion step necessary to form actual liposomes (*see*, Declaration at ¶ 16 and Exhibit B). Furthermore, only 0.055% or 0.14% of the input nucleic acid was recovered and encapsulated post extrusion (*see*, Declaration at ¶ 16 and Exhibit B). Particle sizes for all of these extruded samples were all considerably larger than 100 nm (*see*, Declaration at ¶ 16). As Dr. MacLachlan confirms, these results unequivocally demonstrate that the dehydration-rehydration-extrusion methods set forth in Martin *et al.* do ***not*** produce liposomes that encapsulate plasmid DNA (*see*, Declaration at ¶¶ 16-17). Thus, Martin *et al.* does not anticipate the presently claimed liposomes encapsulating a nucleic acid-condensing agent complex.

In view of the foregoing remarks, Applicants urge the Examiner to withdraw this aspect of the rejection under 35 U.S.C. § 102(a).

C. Lee *et al.* II

Claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Lee *et al.* (*J. Biol. Chem.*, 271:8481-8487 (1996)) ("Lee *et al.* II"). Applicants respectfully traverse.

The Examiner alleges that Lee *et al.* II discloses compositions containing a condensed nucleic acid encapsulated within a liposome (*see,* Office Action at page 5). In response, Applicants assert that Lee *et al.* II fails to teach all of the elements of the claimed invention.

As previously explained by Dr. MacLachlan, Lee *et al.* II discloses nucleic acid-liposome ***complexes*** that are the same as or similar to the complexes disclosed in Lee *et al.* I (*see*, Declaration at ¶ 11). Specifically, Lee *et al.* II describes lipoplexes, *i.e.*, ***complexes*** between the liposomes and nucleic acid-condensing agent which are formed by mixing preformed anionic liposomes with nucleic acid-polylysine complexes in deionized water (*see,* Declaration at ¶ 11). As discussed above in connection with the rejection of the claims as allegedly anticipated by Lee *et al.* I and as explained by Dr. MacLachlan, one of skill in the art would appreciate that mixing preformed liposomes with nucleic acid-polylysine complexes in an

aqueous solution would result in the formation of lipoplexes, and ***not*** liposomes fully encapsulating a nucleic acid (*see,* Declaration at ¶ 11). Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid complexes of Lee *et al.* II also do not comprise a nucleic acid fully encapsulated in a liposome. Accordingly, Lee *et al.* II does not anticipate the presently claimed invention.

In view of the foregoing remarks, Applicants urge the Examiner to withdraw this aspect of the rejection under 35 U.S.C. § 102(a).

III. REJECTION UNDER 35 U.S.C. § 103(a)

The claims have been rejected in various combinations under 35 U.S.C. § 103(a) over a number of different references. Each of these rejections is traversed in detail below.

To establish a *prima facie* case of obviousness, three basic criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference must teach or suggest all the claim limitations. MPEP § 2143. *See also, In re Rouffet,* 47 USPQ2d 1453 (Fed. Cir. 1998). The court in *Rouffet* stated that "even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination." *Rouffet* at 1459. The court has also stated that actual evidence of a suggestion, or teaching, or motivation to combine is required and the showing of a suggestion, or teaching, or motivation to combine must be "clear and particular." *In re Dembiczak,* 50 USPQ2d 1614, 1617 (Fed. Cir. 1999).

A. Lee *et al.* I or Lee *et al.* II

Claims 11-14, 26-28, 30-31, 42, 52-53, 56, 58, and 62-63 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lee *et al.* I or Lee *et al.* II. Applicants respectfully traverse.

In making this rejection, the Examiner acknowledges that neither Lee *et al.* I nor Lee *et al.* II teaches or suggests diameters of the condensing agent-nucleic acid complex, the claimed lipid:nucleic acid ratios, or the addition of the condensing agent in stages or the addition

of two condensing agents, but concludes that each of these parameters would be obvious in view of Lee *et al.* I or Lee *et al.* II (*see,* Office Action at pages 6-7). However, as discussed above in connection with the rejections of the claims under 35 U.S.C. § 102(a) and as previously explained by Dr. MacLachlan, neither Lee *et al.* I nor Lee *et al.* II discloses or even suggests the presently claimed liposomes encapsulating a nucleic acid-condensing agent complex (*see,* Declaration at ¶¶ 8, 9, 11, and 18). Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* I or Lee *et al.* II. Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

B. Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Holland *et al.*

Claims 17-22, 28-29, 45-48, 53-54, 60, and 63-64 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Holland *et al.* (U.S. Patent No. 5,885,613). Applicants respectfully traverse.

In making this rejection, the Examiner acknowledges that none of Lee *et al.* I, Lee *et al.* II, or Martin *et al.* discloses PEG-ceramide, but cites Holland *et al.* as disclosing liposomal formulations comprising PEG-ceramide, and concludes that the presently claimed liposomal compositions would have been obvious over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Holland *et al.* (*see,* Office Action at pages 7-8).

As discussed in detail above and as previously explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see,* Declaration at ¶ 7). In contrast to the presently claimed invention, Lee *et al.* I, Lee *et al.* II, and Martin *et al.* each disclose nucleic-acid lipid ***complexes*** (*see,* Declaration at ¶¶ 9-11). The disclosure of Holland *et al.* of PEG-ceramide does not remedy the defect in any of these references. As explained by Dr. MacLachlan, Holland *et al.* discloses the use of PEG-ceramide in a nucleic acid lipid ***complex*** (*see,* Declaration at ¶ 13). More particularly, Holland *et al.* states:

> Cationic lipids have been used in the transfection of cells in vitro and in vivo. . . .The efficiency of this transfection has often been less than

> desired, for various reasons. ***One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.***
>
> *See,* column 12, lines 28-39 of Holland *et al.* (emphasis added).

Thus, the teachings of Holland *et al.* are clearly directed to forming nucleic acid-cationic liposome ***complexes***, which are structurally and functionally different from the presently claimed liposomes, wherein the nucleic acid-condensing agent complex is encapsulated in the liposome and is resistant in aqueous solution to degradation with a nuclease (*see*, Declaration at ¶ 13). Moreover, as Dr. MacLachlan has explained, the dehydration-rehydration-extrusion methods set forth in Holland *et al.* cannot be used to encapsulate a nucleic acid in a liposome (*see*, Declaration at ¶¶ 16-17). Thus, the cited references, alone or in combination, do not teach or suggest the presently claimed liposomes ***encapsulating*** a condensing agent-nucleic acid complex. Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Holland *et al.* Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

C. Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Lisziewicz *et al.*

Claims 8-10, 23-25, 39-40, 50-51, and 61 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Lisziewicz *et al.* (U.S. Patent No. 6,420,176). Applicants respectfully traverse.

In making this rejection, the Examiner acknowledges that none of Lee *et al.* I, Lee *et al.* II, or Martin *et al.* discloses the use of polythethylenimine, but cites Lisziewicz *et al.* as disclosing polyethylenimine as a nucleic acid condensing agent, and concludes that the presently claimed liposomal compositions would have been obvious over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Lisziewicz *et al.* (*see,* Office Action at page 9).

As discussed in detail above and as previously explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see*, Declaration at ¶ 7). In contrast to the presently

claimed invention, Lee *et al.* I, Lee *et al.* II, and Martin *et al.* each disclose nucleic-acid lipid ***complexes*** (*see*, Declaration at ¶¶ 9-11) . As explained by Dr. MacLachlan, the disclosure of Lisziewicz *et al.* of polyethylenimine (PEI) does not remedy the defect in any of these references (*see*, Declaration at ¶ 14). If anything, Lisziewicz *et al.* teaches away from the use of PEI. Specifically, as Dr. MacLachlan clarifies, Lisziewicz *et al.* compares the efficiency and toxicity of PEI and PEI-mannose as a condensing agent and demonstrates that relative to PEI mannose, PEI (1) is more toxic; (2) requires more DNA to neutralize; and (3) is less efficient for transfection (*see*, Declaration at ¶ 14). Thus, one of skill in the art would not have been motivated to use PEI in view of the disclosure of Lisziewicz *et al.* Even if Lee *et al.* I, Lee *et al.* II, or Martin *et al.* were combined with Lisziewicz *et al.*, the combination would not lead to the presently claimed invention because none of the cited references, alone or in combination, teaches or suggests condensing agent-nucleic acid complexes ***encapsulated*** in a liposome. Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in view of Lisziewicz *et al.*

Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

D. Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in combination with Papahadjopoulos *et al.*

Claims 65-66 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in combination with Papahadjopoulos *et al.* (WO 98/20857). Applicants respectfully traverse.

In making this rejection, the Examiner acknowledges that none of Lee *et al.* I, Lee *et al.* II, or Martin *et al.* discloses the use of reverse phase evaporation or detergent dialysis to prepare liposomes, but alleges that Papahadjopoulos *et al.* describes such methods for making liposomes (*see,* Office Action at page 10).

As discussed in detail above and as previously explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see*, Declaration at ¶ 7). In contrast to the presently

claimed invention, Lee *et al.* I, Lee *et al.* II, and Martin *et al.* each disclose nucleic-acid lipid ***complexes*** (*see*, Declaration at ¶¶ 9-11) . Moreover, as explained by Dr. MacLachlan, Papahadjopoulos *et al.* does not remedy the defect in any of these references (*see*, Declaration at ¶ 15). Papahadjopoulos *et al.* discloses nucleic acid-lipid ***complexes*** formed by mixing preformed liposomes with nucleic acids, which leads to the formation of lipoplexes, *i.e.*, complexes between the nucleic acids and liposomes, but will ***not*** lead to encapsulation of the nucleic acid in the liposomes (*see*, Declaration ¶¶ 9 and 15). In fact, the disclosure of Papahadjopoulos *et al.* explicitly states that the methods described therein are used for forming ***complexes*** between preformed liposomes and nucleic acids and does not disclose or suggest encapsulating nucleic acids in liposomes using detergent dialysis or reverse phase evaporation (*see*, Declaration at ¶ 15). Thus, the cited references, alone or in combination, do not teach or suggest condensing agent-nucleic acid complex ***encapsulated*** in a liposome. Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* I, Lee *et al.* II, or Martin *et al.* in combination with Papahadjopoulos *et al.*

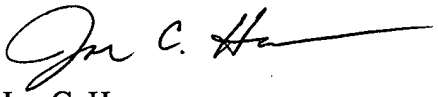
Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



Joe C. Hao
Reg. No. 55,246

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 925-472-5000
Fax: 415-576-0300
JCH:jch
60961111 v1